**1. Introduction**

Probiotics refer to “live organisms which when administered in adequate amounts confer a health benefit to the host” (FAO/WHO, 2006). Probiotics are health-promoting bacteria and also considered as next-generation bio-therapeutics in the field of gut microbiomics (Singh et al., 2019). Apart from enhancing the gut functionality, probiotics also involves in various other health benefits such as brain functioning, boosting immunity, reducing cholesterol, and promoting metabolic homeostasis through their biological mechanisms in the body. The exact mechanism of probiotics is not fully known, but it produce postbiotics such as short-chain fatty acids, enzymes, lactic acid, and also secrete antimicrobial peptides (Pickard et al., 2017). Antibiotic/ antiviral drugs kill beneficial bacteria along with germs; hence to nourish the gut microbiome probiotic supplementation is necessary. “Probiotic Supplements Market - Global Outlook and Forecast 2020-2025” reported that the growth in immune health concerns among people leads to an elevation inthe market growth of the probiotic supplements during the COVID-19 pandemic (Market Research, 2020). The recent report on “Probiotics Market - Growth, Trends, and Forecast” forecasted the global probiotics market to reach USD 76.85 billion by 2024, registering a CAGR of 8.15% during the forecast period between 2020 and 2025 (Mordor Intelligence, 2020). The report also indicated that the bacteria market would grow at the fastest CAGR, owing to the growing demands for prominent applications in fortifying foods with probiotics.

The science of probiotics covers aspects from the field of microbiology to food processing and has found applications in various fields such as nutraceuticals and functional foods, therapeutics in dental care, skincare, oncology, gastroenterology, immunology and psychoneuroendocrinology (Singh et al., 2017). Apart from other delivery systems, in general, probiotics are orally administered and are commercially available in two major forms: functional foods (powders, juices, and incorporated foods) and drug supplements (capsules, tablets). Usually, people prefer food over medicines/drugs considering the hedonistic aspects of food ingestion (Meybodi & Mortazavian, 2017). It has been reported that the viability count of probiotics should be at least 106 CFU/g throughout the product’s shelf-life to achieve the desired therapeutic effects when consumed (Neffe-Skocińska et al., 2018). However, the stability of probiotics is the most desirable concern for targeted colon delivery when ingested orally. It is necessary to maintain its viability during gastro-intestinal (GI) transit to promote their efficacy.

The targeted delivery system helps in selective site-specific delivery of drugs/ therapeutic agents to the target site. This system can also be applied for probiotics (Dodoo et al., 2017). Encapsulation of probiotics providing the protection layer made up of encapsulating material which stabilize the probiotics during processing, storage, and at the site of action by enhancing stress resistance (Douillard & de Vos, 2019). Thereby, the encapsulation process also imparts in targeted delivery. The focus of this review is to provide a complete understanding of the need for encapsulating the probiotics, their impacts on targeted delivery, *in-vitro* and *in-vivo* methods to confirm the targeted action, market status of encapsulated probiotics and economic feasibility for commercialization.

**2. Encapsulation of probiotics: need and critical considerations**

Probiotics ingested through the oral route encounters several stress environments in the alimentary pathway. For instance, the human digestive system has variable pH levels. Approximately, the mouth has a pH of 6-7, the stomach has a lower pH of 1-3, and the pH ranges from 6 to 7 in the small and large intestines (Cieplak et al., 2018). Owing to the low pH conditions in the stomach and the high bile salt content in the small intestine, the viability of probiotic microorganisms that reach the large intestine (colon-target site of action) is a challenge.

Accordingly, appropriate protection of probiotics is vital during the development of any functional probiotic food product. Apart from the conditions of the human body, other factors such as processing temperature, pH of encapsulating/ matrix material, the oxygen level in the product, presence of other competing bacteria, and toxicity of metabolites have implications on probiotic viability (Terpou et al., 2019). In this context, during storage, the temperature and the moisture content of the products are major factors to be considered. Moreover, rehydration and solubility behavior of dried probiotics related to their survival and revival (Broeckx et al., 2016).

Various technologies have been developed to improve the external stress tolerance by probiotics, thereby enhancing colonization of gut microbiota through food matrix modifications and by engaging process engineering approaches. In this regard, food matrix selection and formulation are critical considerations in terms of technological performance and probiotic stability (Pop et al., 2020; Soares et al., 2019; Sun et al., 2020).

The encapsulation of probiotics as powder formulations can protect these live micro-organisms and improve their stability and offer benefits in terms of targeted delivery (Douillard & de Vos, 2019). Importantly, in the process of optimizing an encapsulation methodology for probiotics, microbial stability, functionality, safety, efficacy, and targeting ability must be established before and after the encapsulation process (Terpou et al., 2019). Fig. 1 explains various screening sections involved in the evaluation of probiotics for food use as per FAO/WHO guidelines. To maintain the characteristic features of probiotics, an encapsulation approach should consider the following aspects.

1. Stability of probiotics: Assurance for improved retention of the viability of encapsulated probiotic bacteria;
2. The functionality of probiotics: Functional aspects such as resistance to gastric acids, bile salts and digestive enzymes, antimicrobial activity against potential pathogens, adhesion to mucus, aggregation, and other potential characteristics should be retained after encapsulation;
3. Safety and efficacy: The probiotic strain should be safe, free from contamination, non-toxicogenic, retention of therapeutic efficiency after encapsulation;
4. Targeting ability: Improved tolerance towards environmental stress and the ability to target the colon for the enhancement of gut microbiota and beneficial health effects.

Various techniques have been explored for the encapsulation of probiotics (Fig. 2). Though the focus of all such methods is to protect probiotic viability/stability, the concept of each technique is unique and has direct implications on the product quality. Processing conditions are considered as the major factors that are responsible for the retention of quality and viability of encapsulated probiotics. Further, different wall materials have been explored as protecting/coating agents for the encapsulation of probiotics. They are sourced from dietary fibers, polysaccharides, proteins, and other synthetic polymers. Typically, the selection depends on their functionality, film-forming ability, stability, solubility, digestibility, and releasing properties. To attain the desired properties, a combination of wall materials or the addition of emulsifier/ filling agents can also be employed.

**3. Methods to confirm the targeted delivery and viability of encapsulated probiotics**

Encapsulation of probiotics can protect cell viability until it reaches the colon, allowing improved effectiveness of probiotic functions after reaching the distal part of the intestine (lower GI tract). Confirmation of the delivery of probiotics can be done initially by *in-vitro* studies using simulated static *in-vitro* digestion fluids or dynamic *in-vitro* digestion system (simulator). These can provide a proof-of-concept for targeted delivery. Further, *in-vivo* studies on the delivery of probiotics are needed to have a realistic view of the delivery and the need for extended research on health effects, efficacy, and safety.

***3.1. Static in-vitro digestion studies***

*In-vitro* digestion studies can be performed using simulated digestion fluids such as simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF); nevertheless, these sparingly mimic the digestion process (Fig. 4).

The efficiency of dual-process microencapsulation of *L. plantarum* on the survival of probiotics under simulated GI conditions was evaluated by de Almeida Paula et al. (2019) and reported that the cell viability was observed to be high (80.4%) in microencapsulated *L. plantarum* as compared with free cells (25%). The survival of encapsulated *L. plantarum* (MTCC 5422) was evaluated by Rajam et al. (2012) under SGF and SIF conditions, separately. In both fluids, denatured whey protein isolate (DWPI) encapsulated cells showed better stability than whey protein isolate (WPI). Also, it was reported that the synergistic effect of DWPI and sodium alginate wall matrix could deliver probiotics with high cell survival rates. In another study, FOS was used along with WPI and DWPI for the encapsulation of *L. plantarum* (MTCC 5422), resulting in more viable cells under simulated gastric conditions (Rajam & Anandharamakrishnan, 2015). This is because of the barrier properties offered by whey protein (WP), which does not allow diffusion of the gastric medium into the cells, thereby retaining viable cell counts.

Afzaal et al. (2019) explored the effect of encapsulation on the stability of probiotics (*L. acidophilus*) under simulated GI conditions and observed that non-encapsulated probiotics had 7 log reduction as compared with 3 log reduction in the cell viability of the encapsulated form. In a different study with *L. acidophilus* and *L. casei*, the microencapsulated probiotics (*L. acidophilus* and *L. casei*) were incorporated into freeze-dried banana powder and its survivability under simulated GI conditions (for 90 minutes) was found to be 7.05±0.1 log10CFU/g and 5.48±0.1 log10CFU/g for *L. acidophilus* and *L. casei,* respectively (Bora et al., 2018). In another report, Eckert et al. (2018) concluded that encapsulation with whey-alginate-pectin (WAP) was more effective than whey permeate-alginate-pectin (WPAP) based on observations made on the stability of probiotics under GI conditions. This can be attributed to the strong bonds formed between WAP and bacteria. These researchers also reported a reduction of 3 and 2 log cycles from initial cell count at pH of 2.5 and 3, respectively under simulated GI conditions. Silva et al. (2018) reported that alginate-shellac encapsulating material provided better survival of *L. acidophilus* LA3 in simulated GI fluids because of the resinous nature of shellac. Similarly, Poletto et al. (2019) have studied the influence of different prebiotic materials (inulin, rice bran, alginate, and Hi-maize) on the survival of encapsulated *L. acidophilus* and reported that all these prebiotic materials provided improved protection and enhanced stability of probiotics under simulated GI conditions.

The efficiency of solid/oil/water emulsions prepared with sugar beet pectin as a delivery system for spray-dried *L. salivarius* (NRRL B-30514) was evaluated and observed the increase in cell viability in simulated GI conditions when the secondary emulsion was cross-linked with calcium (Zhang et al., 2016). Recently, Yoha et al. (2020) studied the effect of encapsulation methods (spray drying and spray-freeze drying) on the survivability of *L. plantarum* NCIM 2083 under simulated oral-GI conditions. They concluded that free cells lose their viability, whereas encapsulated probiotics retained. In another study, Yoha et al. (2019a) evaluated the stability of refractance window dried and freeze-dried synbiotics under oral-GI conditions. Their results indicated that refractance window dried synbiotics showed similar efficiency in cell survivability with freeze-dried synbiotics.

***3.2. Dynamic in-vitro digestion studies***

Static models can only represent the biochemical processes in the GI tract, but fails to provide the dynamic environment such as constant physical forces (axial and shear) on the stomach, gastric emptying, synchronized contractions of involuntary muscles for the intestinal peristaltic movement, feedback mechanisms, and the effects of meal and resident microflora. To address these challenges dynamic *in-vitro* digestion models have been developed.

*3.2.1. Mainville’s model (IViDiS)*

A dynamic model of the human upper GI tract mimics the events of the upper GI tract transit. This model consists of a stomach (gastric) and duodenum reactors which can be used for validation of the survival of probiotic bacteria isolated from humans, animals, and fermented dairy products. This model considering the pH variations in the presence of meal in the stomach and bile salt in the small intestine. Especially, it demonstrates the impact of meal as well as encapsulation matrix on the viability of probiotics (Mainville et al., 2005).

Reid et al. (2005) evaluated the stability of extrusion-based encapsulated probiotics using this model. They have reported that at the end of 90 min under GI conditions, free cells significantly lose their viability (~ 4 log reduction), whereas micro-entrapped cells maintain their viability even after 90 min. The study concluded that the Ca2+ induced WPI gelation could protect the cells in the upper GI tract and deliver towards the colon. Following this study, Tompkins et al. (2011) studied the impact of meals on a probiotic during transit through the same model. They observed that the milk with 1% milkfat and oatmeal-milk gruel showed better survival of probiotics than apple juice or spring water and concluded that the protein content of the meal was probably not very important for the survival of the bacteria, unlike the fat content. Further, the study highlighted that non-enteric coated probiotics should be taken with or just before a meal containing some fats. More recently, Aragón-Rojas et al. (2020) investigated the effect of the carrier material (culture medium and culture medium with 0.6:0.4 ratio of maltodextrin (MD):sweet whey), drying technology (spray-drying and freeze-drying) and dissolution media (water and milk) on the viability of *L. fermentum* K73 during simulated GI transit in this model. They observed that the viability of probiotics was not dependent on the drying technology used and the culture medium as a carrier material provided the highest viability with water or milk (both dissolution media) than the culture medium with MD and sweet whey. Further, cell survival was found to increase when digested with milk as the medium. Though this model is more realistic for the validation of probiotics viability, it is not suitable to evaluate the fuctional properties such as mucus adhesion, aggregation effect, and the impact of resident microflora in the gut (Mainville et al., 2005).

*3.2.2. Simulated Human Intestinal Microbial Ecosystem (SHIME®)*

The SHIME® is a computer-controlled simulated human GI model. This model consists of five reactors which simulates from the stomach to the decending colon of large intestine. Stomach reactor mimics the acidic environment with pepsin digestion, small intestine reactor provides the bile conditions for digestive processes and the three reactors of the large intestine mimics the differential regions (ascending, transverse, and descending) of colon which helps to study the microbial processes. Urbanska et al. (2007) evaluated the targeted delivery of alginate-chitosan microencapsulated *L. acidophilus* using SHIME® and observed that the cell viability was retained about 8.37 log10CFU/mL and 7.96 log10CFU/mL after the exposure of gastric (2 h) and intestinal (6 h) conditions, respectively. Thereby, they confirmed the successful delivery of probiotics at the target site. Pham and Mohajeri (2018) highlighted the applications of SHIME® model for the screening of probiotics and prebiotics. Later, Patrignani et al. (2019) studied the potential of *L. crispatus* BC4 along with squacquerone cheese for the prevention of gynecological infections in women. They have evaluated GI stability using SHIME® model and the study confirmed that the viability of probiotic strain was more significantly affected by the low pH of the stomach; whereas, the strain was observed to be resistant towards bile salts and pancreatic juices.

*3.2.3. SIMulator of the Gastro-Intestinal tract (SIMGI®)*

SIMGI® is a multi-compartmental GI model designed to simulate the digestion process in the stomach, small intestine, and large intestine (with ascending, transverse, and descending colon compartments). Yao et al. (2020) highlighted that the SIMGI® system could handle the proliferation of colonic microbiota. Gil-Sánchez et al. (2020) studied the impact of *L. plantarum* CLC17 supplementation in polyphenol metabolism. They used the SIMGI® model for simulated GI digestion and the results inferred that the strain could be successfully delivered in compartments of the colon region. Cueva et al. (2019) studied the impact of silver nanoparticles on human gut microbiota using the SIMGI® model and reported that nanoparticles do not affect the metabolic activity of human intestinal microbiota.

*3.2.4. The Artificial Colon (ARCOL)*

ARCOL is a mono-compartmental model that simulates the colon (large intestine) digestion. It can be employed with any other dynamic upper GI system. The composition of intestinal microbiota and its activity can be studied using the ARCOL model. Moreover, the dialysis fibers present in this model mimics the passive absorption of microbial metabolites (Dupont et al., 2019).

*3.2.5. The TNO Gastro-Intestinal Model (TIM)*

TNO system has two models TIM -1 (consists of stomach, duodenum, jejunum, and ileum) and TIM -2 (large intestine). Marteau et al. (1997) first studied the survival of lactic acid bacteria using TIM-1 and reported that it can be used for the validation of probiotics viability during GI transit. Blanquet-Diot et al. (2012) investigated the influence of biopharmaceutical compounds on the survival of probiotic yeast using TIM -1 with ARCOL and observed that two-fold increase in strain survival because of food-matrix intake along with yeast-HPMC capsule, which delayed the release of yeast. Cordonnier et al. (2015) used TIM -1 with ARCOL dynamic simulators to study the survivalkinetics of *S. cerevisiae* CNCM I-3856 and its influence on intestinal microbiota. Results showed a high survival rate of probiotic strain in the upper gastrointestinal tract whereas the strain was more sensitive to colonic conditions. Venema et al. (2019) studied the survival of multilayer-coated probiotic strains (*L.gasseri* PA 16/8, *B. longum* SP 07/3 and *B. bifidum* MF 20/5) using TNO GI model (TIM-1 system) – the *in-vitro* model of the stomach and small intestine. They have reported that the gastric survival percentage of *Bifidobacteria* and *L. gasseri* at 72% and 53%, respectively were delivered to the small intestine. Thus, the enteric coating of probiotic strains offered 20-40 fold increased delivery of viable cells as compared with uncoated probiotic strains.

*3.2.6. Gastro-Intestinal Tract Simulator (GITS)*

GI tract simulator is a single bio-reactor simulates human GI tract conditions. Sumeri et al. (2008) studied the survival of different probiotic bacteria (*L. acidophilus* La-5, *L. johnsonii* NCC533, *L. casei* strain Shirota, and *L. rhamnosus* GG) using GITS and reported that *L. acidophilus* La-5, and *L. johnsonii* NCC533 exhibits more resistant to bile salts; whereas, *L. casei* strain Shirota, and *L. rhamnosus* GG showed 6 log reduction in their viability.

*3.2.7. Gastro-Intestinal Digestive Simulator (GIDS)*

GIDS - a semi-dynamic *in-vitro* GI model, its mimics “fasted-mode” i.e., digestion in an empty stomach – it did not contain digestive fluid before sample addition. Salivary fluid manually added outside before feeding into the stomach reactor to mimic the oral digestion. Adouard et al. (2019) have developed this model specifically for probiotics and established to assess probiotic viability throughout the human GI tract. This system was initially validated only for fermented milk products and further used for assessing the species (both inter and intra) variability among 16 different bacterial strains of *B. animalis, B. breve,* and *L. paracasei* species. After simulated digestion, the digesta has to be subjected to qPCR and flow cytometry for the quantification of specific viable strains. Adouard et al. (2019) reported that the GIDS system provides valid insights for the identification of targeted bacterial strains to reach the colon.

*3.2.8. Customized dynamic in-vitro digestion systems*

The GI tolerance of encapsulated lactic acid bacteria was studied by Moumita et al. (2017) using a customized *in-vitro* gastrointestinal model. Free and encapsulated forms of lactic acid bacteria were subjected to the digestion process and observed that the encapsulated *L. acidophilus* NCIM 2660 showed increased resistance as compared with its free form. Whereas, *L. bulgaricus* NCIM 2056 and *L. fermentum* NCIM 2156 did not show any significant difference in resistance between encapsulated and free cells. These results concluded that the resistance/ tolerance against stress conditions are strain-dependent apart from the encapsulation. Gbassi et al. (2011) investigated the study on the release of *L. plantarum* strains encapsulated/immobilized in the alginate-WP beads. *In-vitro* GI model was designed to find the release and the viability of probiotic strains with an incubation period of 10.25 h (consists of 5 compartments - 2 h stomach, 0.25 h duodenum, 3 h jejunum, 4 h ileum, and 1 h caecum) and observed that the release of *L. plantarum* was pH-dependent. The probiotic strains were released in the SGIF (Simulated gastro-intestinal fluid) at a range of pH (6.0-6.5) and therefore the viable probiotics were found in the jejunum compartment of the small intestine.

*3.2.9. Other dynamic in-vitro digestion systems*

Parthasarathi et al. (2018) developed an engineered small intestinal system to study the intestinal absorption and perfusion processes with the interference of the mucosal layer. It consists of a perfusion chamber connected with a peristaltic pump, pH meter, donor, receiver, and buffer circulation compartments. Small intestine from small animals (rat/ chicken) can be fitted with this model, which mimics the exact *in-vivo* model. Parthasarathi et al. (2018) used rat small intestine in this engineered small intestinal system to study the intestinal permeability of bioactive compounds and reported that the developed system is well-fitted for passive diffusion of intestinal permeability. This approach was used by Jayan et al. (2019) to study the bioavailability of nano-encapsulated zein-resveratrol and a modified approach can be extended to probiotics. Several other systems such as the M.I.D.A. (Model of an Infant Digestive Apparatus), Dynamic Gastric Model (DGM), Human Gastric Simulator (HGS), DIDGI***®*** system, and Engineered Stomach and Small Intestinal (ESIN) have been used to evaluate food digestibility, dissolution property, absorption and bioaccessibility of nutrients, including pharmaceutical applications.

***3.3. In-vivo studies on probiotics delivery***

Targeted delivery of layer-by-layer encapsulated *Bacillus coagulans* was evaluated in BALB/c mice and reported that layer-by-layer coatings provided enhanced survival of probiotics (Anselmo et al., 2016). Further, they have conducted mucoadhesion and intestinal colonization studies using porcine small intestine and observed that probiotics were well-adhered with the intestinal tissue at a short time-points through bioluminescence imaging using In Vivo Imaging Systems (IVIS) software. Sharma et al. (2017) evaluated the *in-vivo* targeting efficacy of spray-dried probiotics-embedded 5-fluorouracil microparticles using X-ray transmission radiographic technique in Wistar rats. In this study, the radiopaque marker (barium sulfate) was incorporated along with the formulation for tracking the position of the microparticles at various time points during the *in-vivo* movement in the GI tract. Further, this study proved the colon targeted delivery of probiotics.

In another study, Dinoto et al. (2006) investigated the effects of administration of gelatin-encapsulated *B. breve* JCM 1192T cells in the male WKAH/HkmSlc rats. After the study period, the rats were sacrificed and their cecal contents were analyzed using fluorescence in situ hybridization (FISH) and terminal restriction fragment length polymorphism (T-RFLP) for the identification of the specific bacterial strain. *B. breve* cells were detected only in the group treated with gelatin-encapsulated *B. breve* JCM 1192T and it was quantified about 6.3% of the total cells using FISH analysis. Coelho-Rocha et al. (2018) investigated the survival of encapsulated and non-encapsulated lactic acid bacteria in C57BL/6 mice by oral administration and the intestinal sections of mice were analyzed by confocal microscopy and qRT-PCR (quantitative Reverse Transcription - Polymerase Chain Reaction). The results ensured the presence of viable lactic acid bacteria at different sections of the intestine. Researchers have observed that both the encapsulated and non-encapsulated lactic acid bacteria have a higher relative expression in the duodenum and jejunum sections, whereas in ileum and colon sections the relative expression was only observed in the encapsulated lactic acid bacteria.

Probiotic Pearls™ Acidophilus consists of *L. acidophilus* NCFM and *B. longum* BB536 encapsulated in a bi-layer of gelatin and pectin. Mai et al. (2017) investigated the recovery of this encapsulated probiotic strains (Probiotic Pearls™ Acidophilus) in the fecal sample of human volunteers. They have confirmed the presence of encapsulated probiotic strains using strain-specific PCR (Polymerase Chain Reaction), and the differential effects on overall microbiota composition were identified by16S rRNA gene sequencing. Arioli et al. (2018) evaluated the effect of GI transit on the quantitative recovery of viable *L. paracasei* CNCM I-1572 (*L. casei* DG®) in healthy adults. Fecal samples were analyzed and confirmed the presence of viable *L. paracasei* CNCM I-1572 using two molecular techniques (strain-specific colony PCR used for phenotype identification and qPCR used for genotype identification and quantification). The above examples of *in-vivo* studies can confirm the delivery of probiotics in the target site of action; still, proofs are needed for the evidence of their health claims/efficacy/destination for their wholesome applications.

**4. Evidences for the efficacy of encapsulated probiotics**

Several studies have been corroborated the efficacy of encapsulated probiotics, their bioavailability, and safety aspects. Spray-dried *L. plantarum* HM47 was incorporated into milk chocolate and its effect on the acute oral toxicity was evaluated in Swiss albino mice (Nambiar et al., 2018). Enhanced intestinal lactic acid bacteria count and diminished enteric pathogen count were observed in this study, indicative of colonization of encapsulated probiotics in the colon region. Moreover, the safety aspects of probiotic consumption in mice were studied and results confirmed that there was no adverse effect or mortality. Histopathology studies of mice ileum confirmed the absence of necrosis, or inflammation, or any deteriorative changes in the intestinal epithelial cells. In another study, Ayyanna et al. (2018) reported the anti-inflammatory and antioxidant potentials of sodium alginate coated probiotic beads (*L. mucosae* AN1 and *L. fermentum* SNR1) in both acute and chronic animal models (Wistar rats). The study by Wang et al. (2018) explained the effect of microencapsulated probiotics along with prebiotics in the growth performance, antioxidant potential, and immune functions of broiler chickens. They have observed improvement in serum immunoglobulins/ interleukin levels apart from increased cecal lactic acid bacteria count in treated broiler chickens.

**5. Commercial probiotic fortified products**

Most probiotic food products available in the market contain free cells; whereas, their encapsulated forms come in supplements such as tablets, pills, and capsules. Table 1 lists examples of commercially available encapsulated probiotic fortified products. Though the techniques involved are not clear in most cases, consumers are provided with information on specific ingredients in small-font texts on the product label (de Simone, 2019). Most of these products contain mixed probiotic strains rather than a single strain. Table 2 lists recent patents on encapsulated probiotics and/or their incorporation into the products. It is to be understood that a thorough understanding of the effects of such products need to be explored through research studies, failing which, consumers would lack clarity on selection and usage, particularly for specific health conditions. However, some organizations have systematically reviewed the available evidence and developed recommendations on specific information including appropriate product, dose, and formulation (NIH, 2020).

The World Gastroenterology Organization (WGO) explains that the optimal dose of probiotics depends on the strains present in the product. The organization recommends that clinicians/physicians who advise their patients to use probiotics must specify probiotic strains, doses, duration, and benefits of usage. It is also suggested that consumers check the labels of probiotic supplements for clear guidelines on storage conditions and usage (WGO, 2017). ISAPP advises manufacturers to list the “number of CFUs” of viable cells at the time of manufacture as well as a minimum number of viable cells at the end of the product’s shelf-life, also including “expiration/ use by date” on the product label. It is important that consumers check product labels for the number of CFU at the end of the product’s shelf life and not at the time of manufacture. This is because significant losses in cell viability may occur during the storage period (ISAPP, 2017). Earlier, FDA labeling regulations “21 CFR 101.36” mentioned that dietary supplements containing live microbes must provide information on the quantity in terms of weight of the microorganisms on the label; this cell mass consists of both live and dead microorganisms. Later in 2018, these regulations were amended to consider “the quantitative amount of probiotic ingredients in a dietary supplement to be presented in terms of colony-forming units (CFUs) instead of by weight”. This also explains that the quantity in CFUs measures only the live micro-organisms and must not include inactive/ dead/ non-viable cells (FDA, 2018).

**6. Future scope**

The encapsulation of probiotics is well-studied by many researchers using various methods with different encapsulating materials. Polysaccharides or proteins dominate the field. Lipids are important and occasionally edible fats are added as additives (co-encapsulating materials) for encapsulation of probiotics in emulsification/ electrospraying/ spray drying. Liposomes are self-assembled phospholipids that offer a new system for encapsulation. Liposomal delivery systemscan also be used as pH-responsive delivery of drug/vitamin/ enzyme/ antibody/ antigen/gene, etc. It remains a challenge to encapsulate probiotics using the liposome-approach because of the large size (micro-scale) of probiotics (Sarao & Arora, 2015). However, considering their prospective benefits, liposomal delivery of probiotics and its implications needs to be investigated.

On an application basis, probiotics play a vital role in the treatment of various diseases as discussed in earlier sections of this article. Interestingly, the role of probiotics in viral infections – “anti-viral probiotics” is a new concept in medical sciences. Recently many studies have focused on the use of probiotics for the treatment of acute respiratory tract infections, considering the science involved in intestinal–pulmonary cross-talks through the gut-lung axis (Enaud et al., 2020; Zolnikova et al., 2018). The immunomodulation and prophylaxis mechanisms of probiotics can be used in the treatment/prevention against viral infections. It is known that gut probiotics-mediated immunomodulation up-regulatesthe respiratory mucosal immunity by the secretion of cytokines and thereby prevents respiratory viral infections (Kanauchi et al., 2018). It is established that the probiotic strain *Enterococcus faecium* NCIMB 10415 has anti-viral effects against enteropathogenic coronavirus (Chai et al., 2013). There are several interventional clinical trials reported on the effect of probiotics against respiratory tract infections (NCT01782755, NCT03449459, NCT03636191, and NCT03683927).

Recently, probiotics are being recommended by China’s National Health Commission for patients with COVID-19 infection to maintain the balance of intestinal microbiota and prevent secondary bacterial infection (Gao et al., 2020). Further, clinical trials have been registered under the Chinese clinical trial registry (ChiCTR2000029974) to evaluate the efficacy and safety of live *Clostridium butyricum* capsules and live *Bacillus coagulans* tablets for the treatment of patients affected with the novel coronavirus pneumonia and to study its action mechanism. ISAPP highlights the importance of dealing with harmful microorganisms using other microbes, explaining the concept as “germ warfare” (direct antagonism/non-specific immune effects/ metabolic products). Growing awareness of probiotics and their novel findings may pave the way for solutions to better human health, and the role of encapsulation remains critical.

**7. Conclusion**

This comprehensive review explains the role of probiotics in human health and the need for encapsulating probiotics, to achieve the desired benefits. The need for encapsulation is explained by providing an understanding of the complex pathway and the series of stress-environments of the human oral-GI tract. The encapsulation of probiotics has proven potential in protecting probiotics and facilitating its target delivery. Encapsulation techniques have significant implications on probiotics and some of them have been explored only at lab-level to date. A summary report on available commercial products and technologies involving probiotic foods is presented. Probiotics encapsulation, methods of detection of the targeted delivery, and the effects of encapsulation on the probiotics have been explained with key findings from recent studies, providing an up-to-date resource for researchers and industry professionals in this field. All of these include aspects of challenges in research that must be addressed for taking the technology from the lab to the industry.

**Conflicts of interest**

The authors declare no conflicts of interest.

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**Table 1 Commercially available encapsulated probiotics fortified products**

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| --- | --- | --- |
| Product/ company | Product information | References |
| Agropur cooperative  (Granby, QC, Canada) | Probiotics encapsulated by sodium alginate bead and incorporated into the dairy products | (Champagne & Kailasapathy, 2011) |
| Micropharma Ltd.  (Montréal, QC, Canada) | Sodium alginate bead with multiple surface coatings of poly-L-Lysine and alginate in some dairy products | (Champagne & Kailasapathy, 2011) |
| Probiocap™ technology (Montréal, QC, Canada) | Typical freeze-dried powder granule is coated with lipids using a fluidized bed spray-coating process | (Champagne & Kailasapathy, 2011) |
| UltruBiostix (LosAngeles, CA, USA) and  Vitacel®Prolac  (J. Rettenmaier & Söne, Rosenberg, Germany) | Probiotics encapsulated by soluble and insoluble dietary fiber | (Champagne & Kailasapathy, 2011) |
| wowCAPS®  (GAT Food Essentials, Ebenfurth, Austria) | Water-in-oil-in-water layer | (Champagne & Kailasapathy, 2011) |
| Probio’stick® (Montreal, Canada) | Lipid coated particles (powder form) allow cell release only in the intestine | (Champagne &Kailasapathy, 2011) |
| Cardioviva™  (Micropharma Inc., Montréal, QC, Canada and Danone Research) | Microencapsulated *L. reuteri* culture in a fermented milk | (Champagne & Kailasapathy, 2011) |
| Bificapsulas (Yoplait Inc., Mexico) | Yogurt containing particles of encapsulated *Bifidobacterium* | (Champagne & Kailasapathy, 2011) |
| ProBiotic bites (Barry Callebaut AG, Zurich, Switzerland) | Chocolate bars containing encapsulated probiotics. | (Champagne & Kailasapathy, 2011) |
| Mars® Inc.  (Hackettstown, NJ, USA) | Low-calorie probiotic milk drink | (Champagne & Kailasapathy, 2011) |
| YogActive Plus (Yogactive®, QC, Canada) | YogActiveProbioc Cereal - Probiotics fortified ready-to-eat cereal. Matrix-coated probiotics contain rice, wheat, yogurt, fruit fiber, skim milk powder with strawberry/chocolate flavors. Contains 1 Billion CFU of *L. acidophilus* LA-5 per serving (33 g) | <http://www.vandewaterraymond.com/en/news/15-the-worlds-first-and-only-probiotic-cereal.html> |
| EnCaptimusTM  (AnaBioTMTechnologies Ltd., Cork, Ireland) | Beverages, Gummies, Bars, Baby Foods, Sports Powder, Fruit snacks and Trail mixes | <https://www.anabio.ie/products/encaptimus/> |
| PERKii enhanced probiotics  (University of Queensland & Sunshine State®, Queensland, Australia) | Micro-encapsulated probiotics using Progel™ technology - bottled with billions of *L. casei* in different fruit flavor drinks. | <https://www.perkii.com/our-products>  <https://advance.qld.gov.au/whats-happening/stories-about-innovation/perkii-queensland-drink-thats-good-your-gut-inside-out> |
| BioGain™ /BioKid™/Femina™/BioSport™/Ultima16™ (VelobioticsTM, NY, USA) | The probiotic health supplement contains more than 10 strains of probiotics with whey protein, vitamins, and other supplements. Contains 10 Billion CFU of probiotics per capsule.  CSIR, an African R&D organization developed probiotic encapsulation technology (supercritical carbon dioxide technique for encapsulation) and was licensed to a supplier of health-promoting products under the VelobioticsTM brand name. | <https://velobiotics.com/> |
| FlorAssist® for digestive health(Life Extension®, Fort Lauderdale, Florida) | The dual encapsulated probiotic blend contains glycerin, vegetable cellulose, stearic acid, silica, microcrystalline cellulose, chlorophylin along with living bacterial colonies (*Lactobacillus* and *Bifidobacterium* strains). Each capsule contains 15 billion CFUs. | <https://www.lifeextension.com/vitamins-supplements/prebiotics-probiotics> |
| Flying Embers (Fermented Sciences, Inc. and zümXR®) – (Ventura, California) | Shelf-stable probiotic hard kombucha - contains a probiotic strain of *Bacillus coagulans* SNZ 1969 and the native kombucha bacteria (*Acetobacter*) | <https://www.nuffoodsspectrum.in/news/30/6272/fsi-announces-worlds-first-shelf-stable-probiotic-technology.html> |
| Culturelle® (Cromwell, CT) | Digestive health probiotic capsules contain a minimum 10 billion live cultures of *Lactobacillus rhamnosus* GG (LGG®) | <https://www.culturelleprobiotic.ca/resources/culturelle-the-better-alternative-to-yogurt> |
| PRO15 Probiotics (Cognoa International Inc., Manila, Philippines) | Probiotic food supplement - contains 11 *Lactobacillus* and 4 *Bifidobacterium* Strains, Double microencapsulation technology for protective coating of probiotic strains. | <http://www.pro15probiotics.com/> |
| ProbioFerm (Des Moines, IA, USA) | Durabac™ encapsulation technology. Encapsulated powders of individual probiotics with100 billion CFU/g (*L. acidophilus, E. faecium, P. acidilactici, P. pentosaceus, B. bifidum, B. longum,* etc.) | <https://www.probioferm.com/technology.html> |
| UAB “ProBioSanus” (Active Probiotics, Vilnius, LT) | Cleaning products and cosmetics with encapsulated probiotics | <https://probiosanus.com/en/new-innovative-probiotic-product-line-including-live-encapsulated-probiotic-bacteria/> |
| Ayanda Group As, (Oslo, NO) | Softgel capsules contain probiotic bacteria with omega 3 oil (Fish oil with DHA/ EPA and vitamins) | <https://ayanda.com/products/soft-gels/>  <https://www.expresspharma.in/latest-updates/sirios-ayanda-launches-oil-based-probiotic-softgel-capsules/> |
| R.P Scherer Technologies, Llc., (Carson City, Nevada, US) | Stable softgel capsule contains microencapsulated probiotic bacteria | <https://www.catalent.com/oral-dose/softgel-technologies/rp-scherer-softgel-technology/> |
| Bifa-15™ (Eden Foods, Inc., Clinton, Michigan) | *B. longum* with *Lactobacillus* and oligosaccharide - triple-layer encapsulation - seamless microcapsule delivery system. Contains 3 billion live cells per capsule. | <https://www.edenfoods.com/galleries/images/L702-sellsheet-bifa.pdf> |
| Acidophilus Vcaps®  (Natural Organics Inc., Melville, NY, USA) | Pectin coated *L. acidophilus* contains 40 million viable cells. | <https://naturesplus.com/products/productdetail.php?productNumber=4480> |
| UltraBioticDophilus  (NutriDyn™, Maple Plain, MN, USA) | Soft gelatin capsule containing 2 billion viable freeze-dried *L. acidophilus.* | <https://nutridyn.com/ultrabiotic-dophilus> |
| ProBio-40 (Nutracraft, Beaverton, Oregon, US) | Contains 40 billion viable cells of 4 distinctive strains– *L.acidophilus, B.lactis, L. plantarum, L. paracasei.* | <https://www.nutracraft.com/products/probio-40-probiotics> |
| AB-Biotics  (SantCugat del Vallés, Barcelona, Spain) | Encapsulated forms of probiotics - currently play over 550 strains and different products contain more than one billion CFU/dose. | https://www.ab-biotics.com/wp-content/uploads/2019/06/180713-Investement-Research-GCV.pdf  <https://www.ab-biotics.com/c/probiotic-supplements/> |

**Table 2 List of patents related to encapsulated probiotics and their applications**

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| **Company/ Assignee** | **Title of the patent** | **References** |
| Durkee Industrial Foods Corp., Iselin, N.J. | Encapsulated yeast | US4719114A  United States, 12 January 1988 |
| General Mills, Inc., Minneapolis, MN  (US) | Embedding and encapsulation of controlled-release particles | US6190591B1  United States, 20 February 2001 |
| Balchem Corp., NY, US | Solvent released encapsulated yeast | US6616954B1  United States, 9 September 2003 |
| Societe des Produits Nestle SA and Nestle SA, Vevey (CH) | Probiotic delivery system | EP1482811A1  European Patent Office, 8 December 2004 |
| Commonwealth Scientific & Industrial Research Organisation, Campbell, ACT (AU) | Probiotic storage and delivery | WO2005030229A1  WIPO (PCT), 7 April 2005 |
| Mars Inc., US | Foodstuff | US20050079244A1  United States, 14 April 2005 |
| Canacure Corp., ON, CA | Stable probiotic microsphere compositions and their methods of preparation | US20050266069A1  United States, 1 December 2005 |
| General Mills, Inc., Minneapolis, MN  (US) | Cultures encapsulated with chocolate food products coated with chocolate and methods of preparation | WO2006007470A1  WIPO (PCT) , 19 January 2006 |
| Lallemand S.A., Blagnac (FR)Current Assignee: Danstar Ferment AG | Particles containing coated living micro-organisms, and method for producing same | US7157258B2s  United States, 2 January 2007  Later, 12 June 2009 the patent assigned to Danstar Ferment AG |
| Attune Foods, US | Probiotic food, the process for its preparation and dietary regimen | WO2007081981A2  WIPO (PCT), 19 July 2007 |
| Escola Superior De Biotecnologia, Porto (PT) | Pre-fermented symbiotic matrix based on a cereal suspension with encapsulated probiotics, manufacture process, and corresponding utilization. | WO2008041876A2  WIPO (PCT), 10 April 2008 |
| Etherton Law Group, Llc., US | Method of using topical probiotics for the inhibition of surface contamination by a pathogenic microorganism and composition therefor | US20080107699A1  United States, 8 May 2008 |
| General Mills, Inc., Minneapolis, MN  (US) | Cultures encapsulated with compound fat breakfast cereals coated with compound fat and methods of preparation | US20080305210A1  United States, 11 December 2008 |
| Ganeden Biotech, Inc. US | Baked goods | WO2009029267A1  WIPO (PCT), 5 March 2009 |
| Nizo Food Research B.V., (NL) | Protein-based probiotic encapsulates. | WO2009070012A1  WIPO (PCT), 4 June 2009 |
| Probiotical S.P.A., Novara, IT | Chocolate flavored probiotic supplement | WO2010086705A2  WIPO (PCT), 5 August 2010 |
| DeGama Products, Ltd (Grand Caymon) | Process for preparing bakeable probiotic food | US20100303962A1  United States, 2 December 2010 |
| The Procter & Gamble Company, Ohio, (US) | A method of promoting GI health using a combination of a probiotic microorganism and chocolate | WO2010151637A1  WIPO (PCT), 29 December 2010 |
| DeGama Products, Ltd (Grand Caymon) | Heat resistant probiotic compositions and healthy food comprising them | US20110008493A1  United States, 13 January 2011 |
| Erber AG, Herzogenburg (AT) | Probiotic health or fitness promoting human or animal foodstuff and/or drinking water additive and use thereof | US8101170B2  United States, 24 January 2012 |
| R.P Scherer Technologies, Llc., Carson City, Nevada (US) | Process of manufacturing a stable softgel capsule containing microencapsulated probiotic bacteria | WO 2012021432A2  WIPO (PCT), 16 February 2012 |
| Nestec S.A., Vevey (CH) | A consumable product containing probiotics | US8263146B2  United States, 11 September 2012 |
| Advanced BioNutrition Corporation,  Columbia, MD (US) | Dry food product containing live probiotic | US8460726B2  United States, 11 June 2013 |
| Dow Global Technologies Llc., Michigan (US) | Probiotic-containing particles having improved probiotic stability when in aqueous formulations | WO2013188626A2  WIPO (PCT), 19 December 2013 |
| Centro Nacional De Tecnología Y SeguridadAlimentaria, Laboratorio Del Ebro, Universidad De Navarra, Navarra (ES) | Microparticles for the encapsulation of probiotics, preparation and uses thereof | WO2014006261A3  WIPO (PCT), 9 January 2014 |
| Vita-Herb Nutriceuticals, Inc.,  Placentia, CA (US) | Prebiotic and preservative uses of oil-emulsified probiotic encapsulations | US8846082B2  United States, 30 September 2014 |
| Commonwealth Scientific and Industrial Research Organization (CSIRO) (AU) | Probiotic storage and delivery | US8871266B2  United States, 28 October 2014 |
| University Of Saskatchewan (CA) | Microcapsules containing probiotics and methods of making the same | WO2015019307A1  WIPO (PCT), 12 February 2015 |
| Advanced BioNutrition Corporation,  Columbia, MD (US) | The delivery vehicle for probiotic bacteria comprising a dry matrix of polysaccharides, saccharides, and polyols in a glass form and methods of making same | US8968721B2  United States, 3 March 2015 |
| ProGel Pty Ltd, Brisbane (AU) | Microparticles comprising a probiotic, cross-linkable reagent, a denatured protein, polyol plasticizer, and trehalose | US20150313844A1  United States, 5 November 2015 |
| Goodman Fielder New Zealand Ltd., Auckland (NZ) | Probiotic fortified food products and methods of manufacture | WO2015199552A1  WIPO (PCT), 30 December 2015 |
| Ayanda Group As, Oslo (NO) and Golding, Louise, London (GB) | An improved process for producing a softgel capsule comprising viable probiotic bacteria and a soft gel capsule comprising viable probiotic bacteria having a long shelf life | WO2016038355A1  WIPO (PCT), 17 March 2016 |
| DeGamaBerrier Ltd., Grand Cayman  (KY) | Composition and method for improving stability and extending the shelf life of probiotic bacteria and food products thereof | US20160360777A1  United States, 15 December 2016 |
| Vesale Pharma S.A. (BE) and Brace GmbH (DE) | Microencapsulated probiotic substance and process of manufacture | US9554590B2  United States, 31 January 2017 |
| Massachusetts Institute of Technology, Cambridge, MA (US) | Ph-responsive mucoadhesive polymeric encapsulated microorganisms | US20170165201A1  United States, 15 June 2017 |
| FundacionTecnalia Research & Innovation, Edificio, Derio (ES) | Multilayer probiotic microcapsules | WO2017137496A1  WIPO (PCT), 17 August 2017 |
| PepsiCo, Inc., Purchase, NY (US) and  Massey University, Palmerston North (NZ) | Encapsulation system for the protection of probiotics during processing | US9788563B2  United States, 17 October 2017 |
| Mead Johnson Nutrition Co., Chicago, IL (US) | Probiotic stabilization | US20170296600A1  United States, 19 October 2017 |
| Uab " Probiosanus ", Vilnius (LT) | Composition and method for increase of survival and stabilization of probiotic bacteria in detergent-based compositions of personal hygiene and domestic products | US20180360707A1  United States, 20 December 2018 |
| Hill ' s Pet Nutrition, Inc., Topeka, KS, (US) | Pet Food Compositions Including Probiotics and Methods of Manufacture and Use Thereof | US20190142032A1  United States, 16 May 2019 |
| Centro Nacional De Tecnología Y Seguridad  Alimentaria (ES) and Universidad de Navarra (ES) | Microparticles for encapsulating probiotics, production and uses thereof | US20190192439A1  United States, 27 June 2019 |

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**Fig. 1 Screening sections involved in each stage for the functional and safety aspects of probiotics** (FAO/WHO guidelines)

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**Fig. 2 Techniques for the encapsulation of probiotics**

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**Fig. 3 Various encapsulating agents for probiotics**

E:\Probiotic review uploaded final (09.05.2020)\Fig. 4.tif

**Fig. 4 Schematic diagram of static *in-vitro* digestion conditions**